

5 What is claimed is:

1. A modified DNA cleaving enzyme, comprising:

- (a) at least 35% amino acid sequence identity with T7 Endo I,
- (b) two catalytic centers separated by a β -bridge, and
- 10 (c) at least one mutation in the β -bridge that has an effect of altering enzyme cleavage activity compared to the unmodified enzyme.

2. A modified DNA cleaving enzyme according to claim 1, having
15 reduced toxicity in a host cell permitting over-expression of the DNA cleaving enzyme.

3. A modified DNA cleaving enzyme according to claim 1, wherein the enzyme activity comprises at least one of: cleavage at a
20 cruciform structure on DNA, non-sequence specific nicking, nicking opposite a pre-existing nick site, non-sequence specific DNA cleavage and cleavage of DNA at a site flanking a mismatch base pair.

25 4. A modified DNA cleaving enzyme according to claim 1, wherein the product of the altered enzyme activity is a DNA duplex with a single strand over-hang of less than 11 nucleotides.

5. A modified DNA cleaving enzyme according to claim 1, wherein
30 the altered enzyme cleavage activity further comprises a broadened enzyme specificity compared with the unmodified enzyme.

6. A modified DNA cleaving enzyme according to claim 5, wherein the DNA cleaving activity further comprises cleaving at a mismatch

5 in a duplex where the mismatch can be any of an A, T, G or C bases.

7. An A modified DNA cleaving enzyme according to claim 1,
wherein an alteration in the enzyme cleavage activity of the
10 modified enzyme compared to the unmodified enzyme occurs in a
manganese-containing buffer.

8. A modified DNA cleaving enzyme according to claim 7, wherein
the altered enzyme activity is selected from: maintenance of
15 cleavage activity, reduction of non-specific nuclease activity,
enhanced nicking activity opposite a pre-existing nick site, and a
decreased ratio of nicking to double strand cleavage.

9. A modified DNA cleaving enzyme according to claim 1, wherein
20 the altered enzyme activity occurs in magnesium buffer.

10. A modified DNA cleaving enzyme according to claim 9, wherein
the altered enzyme activity is selected from: an increased ratio of
nicking of a cruciform structure in the DNA relative to double strand
25 cleavage; an increased ratio of cleaved DNA of a cruciform to non-
cleaved DNA; a reduced ratio of non-specific nuclease activity; and
reduction in nicking opposite a preexisting nick site.

11. A modified DNA cleaving enzyme according to claim 1, having
30 enhanced or reduced activity for cleavage a DNA under modified
reaction conditions when compared with the unmodified enzyme.

12. A modified DNA cleaving enzyme according to claim 12, wherein
the modification in reaction conditions are selected from changing

- 5 at least one of :pH, temperature, manganese or magnesium salt concentration of the reaction mixture and time of the reaction.

13. A modified DNA cleaving enzyme according to claim 1, selected from a class of enzymes comprising: gene 3 (enterobacteriophage
10 T7), T7 endodeoxyribonuclease I, Yersinia pestis phage phiA1122 endonuclease, Phage Phi Ye03-12 endonuclease, Phage T3 endonuclease, phage T3 endodeoxyribonuclease, Pseudomonas phage gh-1 endonuclease, pseudomonas putida KT2440 endodeoxyribonuclease I; and Roseophage S101 RP endonuclease I.

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14. A modified DNA cleaving enzyme according to claim 1, wherein the at least one mutation is a mutation at a PA site in the β -bridge.

15. A modified DNA cleaving enzyme according to claim 14, wherein
20 the at least one mutation at the PA site is a substitution of PA or deletion such that the substitution of PA is selected from a single amino acid, a dipeptide, a tripeptide and a tetrapeptide.

16. A modified DNA cleaving enzyme according to claim 15, wherein
25 the at least one mutation in the β -bridge is selected from PA/A, PA/AA, PA/PGA, PA/PAPA, Δ PA, PA/K, PA/G, PA/D and PA/P.

17. A modified DNA cleaving enzyme according to claim 15, wherein the PA dipeptide is located at position 46 and 47 in SEQ ID. No. 13.

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18. A nucleic acid comprising a DNA sequence that substantially corresponds to SEQ ID NO:1 wherein at least one mutation has been introduced in the sequence corresponding to the β -bridge.

- 5 19. The nucleic acid according to claim 18, wherein the mutation occurs at a site which encodes the PA in the β -bridge.
20. The nucleic acid according to claim 19, wherein the mutation at the site that encodes PA is a substitution or deletion such that the substituted nucleic acid encodes a single amino acid, a dipeptide, a tripeptide and a tetrapeptide.
- 10 21. A nucleic acid according to claim 17, wherein the at least one mutation results in an amino acid change selected from PA/A, PA/AA, PA/PGA, PA/PAPA, Δ PA, PA/K, PA/G, PA/D and PA/P.
- 15 22. A vector encoding the nucleic acid of any of claim 18 through 21.
- 20 23. A host cell containing a vector of claim 22.
24. A kit containing at least one of: a modified DNA cleaving enzyme of claim 1, a nucleic acid of claim 18-21, a vector of claim 22, or a host cell of claim 23.
- 25 25. A method for modifying enzyme catalytic activity, comprising:
- (a) selecting an enzyme having two catalytic centers connected by a β -bridge, the catalytic centers being located at reciprocal stereo-geometric positions in the enzyme;
 - 30 (b) changing the reciprocal stereo-geometric position of the two catalytic centers by introducing a mutation into the β -bridge; and
 - (c) modifying the catalytic activity of the enzyme.

5 26. A method of determining whether a DNA substrate has a single nucleotide polymorphism (SNP), comprising:

(a) contacting the DNA substrate with a modified DNA cleaving enzyme according to claim 1; and

10 (b) determining from the cleavage product whether the DNA substrate has the SNP.

27. A method according to claim 26, further comprising: identifying which nucleotide forms the SNP.

15 28. A method according to claim 26, further comprising: identifying the location of the SNP.

29. A method of forming a shotgun cloning library, comprising
(a) incubating a modified DNA cleaving enzyme according to
20 claim 1 with a DNA to form non-sequence specific cleavage fragments of the DNA that are ligatable; the ligatable DNA being capable of insertion into a vector for cloning in a host cell; and
(b) forming the shotgun cloning library.

25 30. A method for mapping nicks in a duplex DNA, comprising;
(a) incubating a modified DNA cleaving enzyme according to claim 1 with the duplex DNA in a manganese-containing buffer;
(b) permitting nicking to occur across from a pre-existing nick site to form fragments of the duplex DNA with single strand
30 overhangs; and
(c) mapping the nicks in the DNA.

31. A method for over-expressing T7 endonuclease 1, comprising

- 5 selecting a host cell according to claim 23 and over-expressing the T7 endonuclease 1.